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Integrated Approach for Evaluating Species and Interindividual Differences in Responsiveness to Dioxins and Structural Analogs

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a ubiquitous environmental contaminant that is produced inadvertently during the synthesis of some organochlorine compounds, such as the chlorinated phenoxy pesticides. It is biologically and ecologically persistent, with an estimated half-life of 7 years in humans. It possesses high acute toxicity in rodents and is a carcinogen, teratogen, and immunotoxin. In chronic bioassays for carcinogenicity, TCDD at a dose of 10 ng/kg/day increases the incidence of liver tumors in female rats, making it one of the most potent animal carcinogens ever tested. A recent study in humans has shown an increase in the incidence of respiratory tract tumors in workers in chlorinated phenoxy herbicide plants. Considerable controversy and uncertainty remain, however, concerning its carcinogenic potency in humans and the reliability of using animal data to predict human risks.

It is generally accepted that most, if not all, of the effects of TCDD require its binding to the *Ah* receptor. In addition to its toxic effects, TCDD produces a number of biochemical effects, such as induction of CYP1A1, downregulation of binding activity of the estrogen and epidermal growth factor (EGF) receptors, and changes in cytokine pathways. These effects suggest that the *Ah* receptor plays an important role in regulating the cell cycle. A number of structural analogs of TCDD, such as the polychlorinated dibenzofurans, also interact with the *Ah* receptor, and they produce the same spectrum of responses as TCDD in animal and cell models. The potency of these compounds is strongly correlated with their binding affinity to the *Ah* receptor. Perhaps the most sensitive marker of exposure to TCDD in both rodents and humans is induction of a specific isozyme of cytochrome P450 (CYP1A1). Our data suggest that humans are at least as sensitive as rats to CYP1A1 enzyme induction (produced by transcriptional activation of the *CYP1A1* gene) and to down-regulation of the EGF receptor. This conclusion is based on data obtained *in vivo* (accidentally exposed humans and rats subjected to a two-stage model of hepatocarcinogenesis) and *in vitro* (incubation of human and rodent lymphocytes with TCDD). There is considerable interindividual variation in human responses to TCDD. Our work on "markers of susceptibility" suggests that both variation in the amount of *Ah* receptor and a mutation in the *CYP1A1* gene may be responsible for this variation. Since TCDD gives negative results in short-term tests for genotoxicity and does not bind covalently to DNA, it can be considered a prototypical chemical for the study of receptor-mediated carcinogenesis

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is produced inadvertently during the synthesis of some organohalogen compounds, such as the herbicide 2,4,5-trichlorophenol, and is formed as a by-product of combustion of chlorine-containing wastes and during the bleaching of paper pulp during paper production. Over the last 20 years, this compound has received considerable attention because it is acutely toxic to experimental animals at very low doses and because of its presence as a trace contaminant in food, water, and soil. In guinea pigs, TCDD has an LD_{50} of 1 μg/kg; other species are considerably less sensitive to its acute toxic effects (*1*).

TCDD and its structural analogs, such as the polychlorinated dibenzofurans (PCDFs), produce a wide spectrum of effects in experimental animals, which include teratogenicity, carcinogenicity, immunotoxicity, and a variety of biochemical effects involving drug-metabolizing enzymes and growth factor pathways (*2,3*). Some of these effects have been observed in humans exposed accidentally or occupationally to TCDD or PCDFs.

This paper deals with the mechanism of action of dioxin, comparative effects in humans and rodents, and dose–

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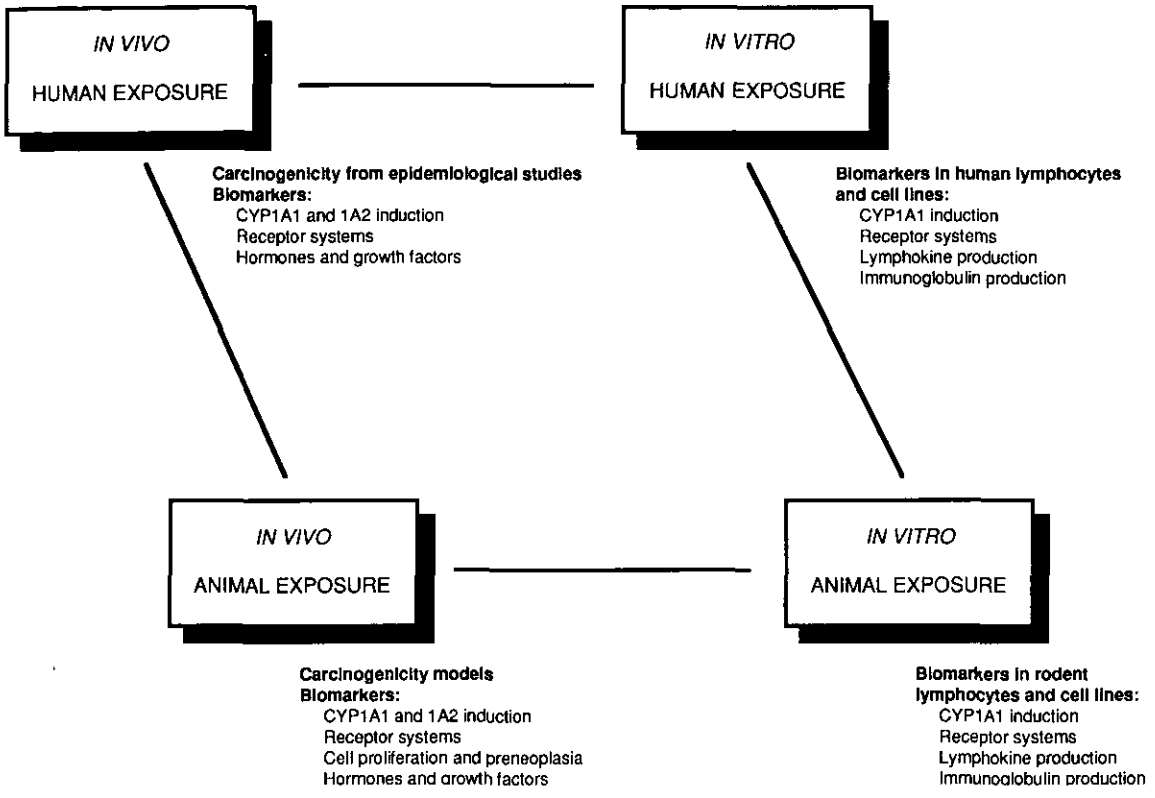

FIGURE 1. Integrative approach for assessment of risk for exposure to dioxins. This approach attempts to incorporate data from exposures to dioxins *in vivo* and *in vitro* in both human and animal studies. PCB, polychlorinated biphenyl; PCDF, polychlorinated dibenzofuran; EGF, epidermal growth factor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; EROD, ethoxyresorufin-*O*-deethylase; AHH, arylhydrocarbon hydroxylase.

response relationships for various effects. These issues are discussed in relation to the potential use of biomarkers (exposure, effect, and susceptibility) in evaluating potential adverse health effects in humans. Shown in Figure 1 is our integrative approach to assimilate data from human and animal studies following exposure both *in vivo* or *in vitro* to TCDD or its structural analogs. End points discussed include: *a*) evidence for carcinogenicity in epidemiological studies of accidentally exposed populations and in carcinogenicity studies in laboratory animals; *b*) biomarkers of exposure, including alterations of biochemical parameters in tissues from exposed human populations and from animal studies; and *c*) biomarkers of susceptibility after exposures of human and murine lymphocytes *in vitro*.

Evidence for Carcinogenicity of Dioxins from Human and Animal Studies

Animal Carcinogenicity Data

There is convincing evidence that TCDD is a multisite carcinogen. In chronic bioassays for its carcinogenicity in rats, tumor incidences were increased at a number of sites. Available studies on TCDD carcinogenesis have been reviewed (*4*) and carcinogenic responses were seen after either long-term or short-term exposures to TCDD in every species studied. Tumors of various types have been induced in several organ systems, and these do not appear to be restricted to the site of application. In rats, TCDD induced neoplasms of the lung, oral/nasal cavities, thyroid, adrenal glands, and liver. In mice, TCDD caused neoplasms of the liver, subcutaneous tissue, and thyroid gland, and lung and thymic lymphomas. In hamsters, it produced squamous-cell carcinomas of the facial skin. Tumors of the integumentary system have been reported after oral (rats and mice), intraperitoneal (hamsters), and dermal (mice) exposures. TCDD is a trans-species (rat, hamster, mouse), trans-strain, trans-sex, multisite, complete carcinogen. It induced carcinogenic effects in laboratory animals at exposures as low as 0.01 μg/kg body weight/day in rat liver and 0.001 μg/kg/day in rat thyroid and is clearly among the most potent of all identified chemical carcinogens (*4*).

TCDD is ineffective in short-term tests for genetic toxicity (*5,6*); moreover, it does not bind covalently to DNA either in *in vitro* or *in vivo*, even when methods are used that can detect one adduct in 10^{11} normal nucleotides (*7*). Therefore, TCDD is generally considered to be a nongenotoxic carcinogen; however, it may exert indirect genotoxic effects, as evidenced by one study in which TCDD

increased the frequency of strand breaks in rat liver nuclei, possibly by increasing iron bioavailability and lipid peroxidation (*8*).

There is strong evidence in experimental animal models that TCDD is a tumor promoter and not an initiator, although it must be kept in mind that the term "tumor promoter" is an operational rather than a mechanistic term. In multistage models for skin and liver cancer (*9–11*), TCDD is a potent promoter but is virtually devoid of initiating activity.

Both hepatocellular carcinomas and adenomas were detected in a two-stage model for hepatocarcinogenesis in rats (a single initiating dose of *N*-nitrosodiethylamine followed by chronic exposure to 100 ng/kg/day TCDD). Interestingly, ovariectomy protected female rat liver from the tumor promoting actions of TCDD (*12*). This finding is consistent with data from chronic bioassays which demonstrated that TCDD produces liver tumors in female but not in male rats (*13*). The carcinogenicity data are also consistent with those on cell proliferation, which showed that TCDD increases cell proliferation rates in intact female rats but not in ovariectomized rats (*12*). In contrast, ovariectomy sensitized rats to the tumor promoting actions of TCDD in lung (*14*). These findings add to the growing amount of evidence that interactions of TCDD with hormonally mediated events are a critical component of the carcinogenic mechanisms of this chemical.

Human Cancer

Some reports in the literature indicate that exposure to phenoxyacetic acid herbicides, chlorophenols, and chlorinated dibenzodioxin contaminants, especially TCDD, may be associated with human cancer, including malignant lymphomas, soft-tissue sarcomas, thyroid tumors, lung tumors, and cancers at other sites (*15*). The International Agency for Research on Cancer (IARC) (*15*) and the National Institute for Occupational Safety and Health (NIOSH) recently completed two pooled cohort studies. The NIOSH registry covered workers with possible exposure to TCDD during the manufacture/formation of 2,4,5-trichlorophenoxy acetic acid and 2,4,5-trichlorophenol. The IARC registry included workers exposed to all types of phenoxy acids and chlorophenols during manufacture and use.

The incidence of thyroid tumors was elevated in the IARC registry, and the risk was highest in workers with probable TCDD exposure. An increased risk for all cancers combined was observed in the NIOSH registry, and the risk increased with duration of exposure and latency. The NIOSH registry showed an increased incidence of respiratory tract cancers. Both the NIOSH and IARC registers reported a statistically nonsignificant increase in the incidence of soft-tissue sarcoma. Statistically significant increases in liver tumor incidences were seen in neither registry, which consisted primarily of men.

The human carcinogenicity data are consistent with the animal data in several respects. First, increased incidences of thyroid tumors were detected in both rats and humans. Second, liver tumor incidence was increased in neither male rats nor human males. Third, increases in lung tumor incidence were evident in the NIOSH registry and in male rats.

Several issues confound human epidemiological studies on the carcinogenicity of TCDD. Exposures in the workplace occur as part of complex mixtures, so it is difficult to ascertain the role of TCDD in increasing tumor incidence. Also, humans are exposed daily to several ubiquitous, persistent structural analogs of dioxin, including the PCDFs and some polychlorinated biphenyls. Therefore, no true control population exists. Furthermore, there is increasing evidence of a great deal of interindividual variation in the magnitude of background exposures.

The environmental contamination episode in Seveso, Italy, in 1976 is a different exposure scenario than that represented by the IARC and NIOSH registries; the individuals in Seveso were exposed primarily to TCDD and not to the numerous other chemicals experienced by the NIOSH and IARC cohorts. Although the follow-up has been only 10–15 years and therefore of insufficient latency, some information on cancer mortality has been published. The standardized mortality ratios were elevated for certain cancers, including soft-tissue sarcoma, Hodgkin's disease, cancers of the gallbladder and bile duct, and thyroid and other endocrine-associated tumors (*16*). These results are based on one to three deaths only, however, making it difficult to establish patterns relating tumor occurrence specifically to TCDD at this time.

Biomarkers for Exposure in Animals and Humans

Animal Studies

We have used a chronic exposure regimen in the context of a tumor promotion model with *N*-nitrosodiethylamine as the initiating agent and TCDD as the promoter in Sprague-Dawley rats to investigate dose-response relationships for biochemical responses and their association with carcinogenic processes. In these experiments, female rats received a single initiating dose of *N*-nitrosodiethylamine followed by chronic exposure to TCDD by gavage at doses ranging from 0.1 to 125 ng/kg/day for 30 weeks. Dose-response relationships for a number of biochemical responses following exposure to TCDD are summarized in Table 1. One of the most sensitive biochemical markers for exposure to TCDD is induction of CYP1A1 in the liver of exposed animals; exposures to as little as 0.1 ng/kg/day appear to produce detectable increases in CYP1A1 activity. Mathematical evaluation of this data indicates that there is no apparent threshold for TCDD-mediated induction of CYP1A1 or CYP1A2 (*17*). Down-regulation of the binding of EGF (epidermal growth factor) to its receptor following TCDD exposure was another sensitive marker for TCDD-mediated effects, exhibiting an ED_{50} of 12 ng/kg/day, which is similar to the ED_{50}s for CYP1A1 and CYP1A2 induction. TCDD-mediated increases in cell proliferation, γ-glutamyl transpeptidase-positive foci, and serum 5′-nucleotidase were less sensitive for detecting TCDD-mediated effects but may be more appro-

Table 1. Comparative dose–response relationships for TCDD-mediated effects in a tumor promotion model (single initiating dose of *N*-nitrosodiethylamine followed by chronic TCDD exposure for 30 weeks).

TCDD-mediated effect	Dose, ng/kg/day	
	Lowest detectable effect	Approximate ED_{50}
CYP1A1 induction	0.1	15
CYP1A2 induction	0.3	20
Cell proliferation	125	ND
Foci[a]	125	ND
5′-Nucleotidase	35	ND
Epidermal growth factor receptor	3.5	12

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; ND, not determined.

[a]Proportion of liver occupied by γ-glutamyl transpeptidase-positive foci.

priate markers for its carcinogenic effects. The concentrations of TCDD in liver were linearly related to the administered dose over the entire dose range.

Human Exposures

In Taiwan during 1979, a widespread poisoning incident occurred due to ingestion of rice oil contaminated with PCDFs. These compounds are structurally similar to TCDD and are thought to share the same mechanism of action: binding to the *Ah* receptor and induction of pleiotropic responses. Placentas from women exposed to contaminated rice oil were analyzed for EGF receptor binding and EGF-stimulated autophosphorylation as well as for CYP1A1 activity; the data are displayed in Table 2 (*18,19*). The placentas from the exposed women showed decreased EGF receptor autophosphorylation activity although the number of binding sites was similar to that in control samples. The number of detectable binding sites for the glucocorticoid receptor was reduced in the exposed women to 42% of that in the control individuals. In rats and mice, glucocorticoid and EGF receptor binding is decreased by TCDD treatment (*20,21*). Exposure to PCDFs resulted in an approximately 100-fold induction of CYP1A1-dependent enzyme (ethoxyresorufin-*O*-deethylase; EROD) activity over that in the unexposed control; however, there was considerable interdividual variation in the response (Table 2). Although CYP1A1 induction occurred only in placentas of women who had ingested the contaminated rice oil, the magnitude of induction in the exposed individuals did not correlate with placental concentrations of polychlorinated biphenyls or of PCDF or with decreased birthweights in offspring of exposed mothers. These studies indicate that humans are at least as sensitive as rats to many of the biochemical effects of TCDD, including enzyme induction (*19*).

Biomarkers for Susceptibility in Humans and Animals

Ah Receptor

Studies carried out largely in rodents indicate that many of the toxic responses elicited by dioxin (including the promotion of tumors) are mediated by a specific intracellular receptor protein (designated the *Ah* or dioxin receptor) (*10,22*). In certain strains of mice, TCDD-mediated induction of CYP1A1 segregates with a single genetic locus (*Ah*) (*23*). The *Ah* locus has been postulated to contain the structural gene for the dioxin receptor. The first published evidence for involvement of the dioxin receptor in TCDD-dependent toxicity was an analysis of the dose-dependency, stereospecificity, and genetic segregation of TCDD-induced thymic atrophy and fetal abnormalities in two prototype inbred mouse strains (*24*). Since publication of that report, many studies have established the involvement of the dioxin receptor in a variety of toxic responses elicited by TCDD, including epidermal hyperkeratinization in mice and humans and tumor-promoting activity in rodents. Most of the existing knowledge is largely correlative, however, and does not provide specific information on how this receptor functions to elicit the biochemical changes in target cells that lead to the observed pathological lesions.

Multiple complementation groups have been identified in the TCDD-dependent induction of CYP1A1, suggesting the involvement of more than one protein in this receptor-mediated response (*25*). Recently, the complementary DNA and part of a gene for a protein (designated *arnt*) required for the cytosol-to-nucleus translocation of the TCDD-dioxin receptor complex in murine hepatoma cells were cloned (*26*). The protein was found to contain significant homology to the consensus sequence for the basic

Table 2. Comparison of placental CYP1A1 induction and polychlorinated dibenzofuran (PCDF) concentrations of women exposed to contaminated rice oil and birthweight of their offspring.

Ethoxyresorufin-*O*-deethylase, pmole/min/mg protein		Birthweight, g		Total PCDF concentration	
Exposed	Controls	Exposed	Controls	Exposed	Controls
34	<1	2900	3500	NA	<0.01
31	<1	2700	3500	0.7	<0.01
63	<1	3000	3250	0.9	<0.01
42	<1	2700	3400	0.5	<0.01
62	<1	3000	3100	NA	<0.01
1	<	3100	3900	0.8	<0.01
43	<1	2700	3500	0.8	<0,01
11	<1	3100	2800	0.6	<0.01
50	<1	2500	3700	NA	<0.01

NA, not analyzed.

helix-loop-helix motif characteristic of proteins that bind DNA as homo- or heterodimers.

Differences in sensitivity to TCDD have been well-documented in mice. At least one determinant resides at the level of signal recognition, i.e., binding of TCDD to the dioxin receptor (*10*). Studies carried out in human squamous-cell carcinoma cell lines (*27*) and in early-passage human thymic epithelial cells derived from different individuals (*28*) provide evidence for potential polymorphism in the human dioxin receptor, which in turn results in differential sensitivity to TCDD.

Exposures of Human and Murine Lymphocytes *in Vitro*

Establishing dose-response relationships for TCDD-mediated effects *in vitro* is one important step in generating data useful in the evaluation of human sensitivity to TCDD. Two reports have been published of dose-response relationships for induction of arylhydrocarbon hydroxylase (AHH) in human lymphocytes. Kouri and Ratrie (*29*) reported an EC_{50} of 8 nM TCDD for induction of AHH activity in mitogen-stimulated peripheral blood lymphocytes while Nagayama et al. (*30*) reported an EC_{50} of approximately 12 nM TCDD in Epstein-Barr virus transformed cell lines. Waithe et al. (*31*) analyzed the physicochemical characteristics of the *Ah* receptor from an Epstein-Barr virus-transformed cell line and demonstrated that the K_d for TCDD binding to the *Ah* receptor was 4.6 nM TCDD. Recently, Neubert et al. (*32*) reported that human peripheral blood lymphocytes are exquisitely sensitive to TCDD-mediated alterations in cell surface marker expression at doses as low as 0.1 pmole. In various human breast carcinoma cell lines, a range of inducibility has been observed for transfected CAT constructs containing the upstream regulatory region of the *CYP1A1* gene the EC_{50} for the response varying from 0.4 to 5.9 nM TCDD (*33*).

To compare the sensitivity of human and murine lymphocytes to TCDD, we analyzed dose–response relationships of lymphocytes for induction of EROD activity: The EC_{50} values and maximal responses for human lymphocytes and murine lymphocytes are shown in Table 3. Exposure of human lymphocytes to TCDD resulted in a dose-dependent increase in EROD activity, with an EC_{50} of 1.8 ± 0.8 nmole TCDD. The response was much greater in human peripheral blood lymphocytes than in murine splenic lymphocytes, although the EC_{50} for the response was similar. Murine lymphocytes responded in a dose-dependent manner to TCDD, with an EC_{50} of 1.3 ± 0.3 nmole. This response correlates well with those to other TCDD-mediated effects on murine lymphocytes. For example, the EC_{50} for inhibition of antibody-producing cells *in vitro* was 7 nM TCDD (*34*). Blank et al. (*36*) also demonstrated that TCDD in doses of nanomoles inhibits antibody synthesis and stimulation of EROD activity in murine lymphocytes. Luster et al. (*37*) showed that exposure of murine B lymphocytes *in vitro* to nanomolar concentrations selectively inhibited antibody production.

Table 3. Ethoxyresorufin-*O*-deethylase (EROD) response of murine and human lymphocytes to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

	EROD activity,[a] *pmole/min × mg*		
Lymphocytes	Baseline	Maximum	EC_{50}, nM TCDD
Human[b]	2.8 ± 2.4	58.1 ± 52.0	1.8 ± 0.8
Murine[c]	0.4 ± 0.3	2.5 ± 0.6	1.3 ± 0.3

[a]C57Bl/6 splenic lymphocytes were isolated and cultured at 1×10^6 cells/mL in RPMI 1640 containing 2% phytohemagglutinin (Gibco, Grand Island, NY), 0.13% pokeweed mitogen (Gibco), 1 mM glutamine, and 5×10^{-5} M β-mercaptoethanol for 3 days at 37°C in a 5% CO_2 atmosphere. Human peripheral blood lymphocytes were isolated using Leucoprep tubes (Beckton Dickinson, Lincoln Park, NJ) by the manufacturers' suggested method. The cells were cultured at 1×10^6 cells/mL in RPMI 1640 containing 2% phytohemagglutinin, 0.13% pokeweed mitogen, and 1 mM glutamine for 3 days at 37°C in a 5% CO_2 atmosphere. Cultured lymphocytes were exposed to various concentrations of TCDD absorbed into fetal calf serum, as previously described (*34*). After 3 days in culture, the cells were assayed for EROD activity, as described by Thompson et al. (*35*).

[b]Data represent mean ± SD for a cohort of 36 individuals; inducibility of EROD activity was replicated at least twice for each individual.

[c]Data represent mean ± SD for five individual experiments.

Recently, Clark et al. (*38*) demonstrated that exposure to such concentrations *in vitro* resulted in the dose-dependent stimulation of tyrosine kinase activity in murine lymphocytes. The finding that both human and murine lymphocytes respond to nanomolar concentrations of TCDD suggests that the two species respond similarly.

Another important consideration for assessing human risk for TCDD-mediated effects is interindividual differences in response, perhaps related to genetic susceptibility. As discussed above, the *Ah* receptor appears to regulate differences in susceptibility to TCDD-mediated effects in various strains of mice. Humans also demonstrate interindividual differences in their responsiveness to TCDD and related compounds. We have analyzed CYP1A1 induction by quantifying EROD activity in lymphocytes from a cohort of 36 individuals. Interindividual variation in maximal induction of EROD activity by TCDD is shown in Figure 2. The responses fall into an essentially bimodal or perhaps trimodal distribution, with approximately 70% of the individuals having a low response and 30% responding with a high maximal EROD response. This finding may be due to genetic differences in the capacity of human cells to be induced by TCDD. A number of investigators have suggested that a high inducibility phenotype for AHH induction may be associated with increased susceptibility to lung cancer (*39–41*). Induction of EROD activity in human lymphocytes *in vitro* may therefore be useful for phenotyping individuals for susceptibility to adverse human health effects following exposure to TCDD or polycyclic aromatic hydrocarbons.

We have attempted to characterize inducibility phenotypes further with restriction fragment length polymorphism (RFLP) genotype analysis. Kawajiri et al. (*42*) recently described an *Msp*I polymorphism in the 3′ noncoding region of the *CYP1A1* gene that was associated with increased risk for lung cancer. Petersen et al (*43*) reported that in one family heterozygosity for this poly-

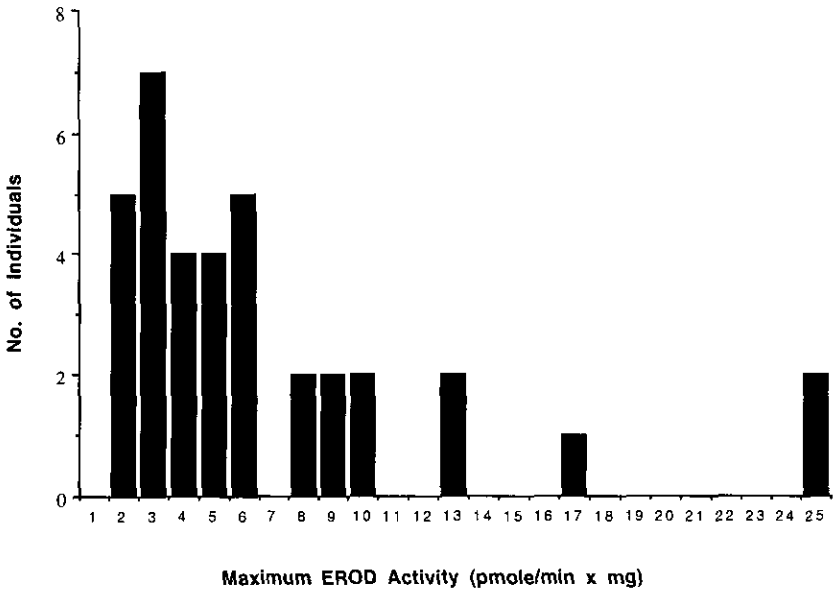

FIGURE 2. Frequency distribution of maximal ethoxyresorufin-*O*-deethylase (EROD) response in TCDD-treated human lymphocytes. Human peripheral blood lymphocytes were cultured and EROD activity measured as described in Table 1. Data are expressed as frequency of individuals responding with maximal EROD induction.

morphism co-segregated with the AHH high-inducibility phenotype. We have compared the EROD inducibility phenotype with the *CYP1A1 Msp*I polymorphism in 19 individuals and found none to be homozygous for the *Msp*I site (MspI$^{+/+}$). In the low-inducibility group 8% ($^1/_{12}$) were heterozygous (MspI$^{+/-}$) while in the high inducibility group 43% ($^3/_7$) were heterozygous for the RFLP. In this small group, there does appear to be an association between the presence of the *Msp*I allele and EROD inducibility; however, the mechanistic link between this polymorphism and EROD inducibility and its relationship to other biological effects such as cancer remain unclear.

In mice, allelic differences in the *Ah* receptor result in strains with different susceptibilities to the toxic and biochemical effects of TCDD (*10,44*). We are analyzing human lymphocytes for levels of *Ah* receptor protein, using photoaffinity ligands for the receptor, to determine if there is an association between high inducibility and *Ah* receptor levels. Figure 3 is an autoradiograph of photoaffinity labeled *Ah* receptor preparations from three individuals. Different amounts of labeled protein were electrophoresed in polyacrylamide gels for each individual. The amount of photoaffinity labeled *Ah* receptor was related linearly to protein concentration under the condi-

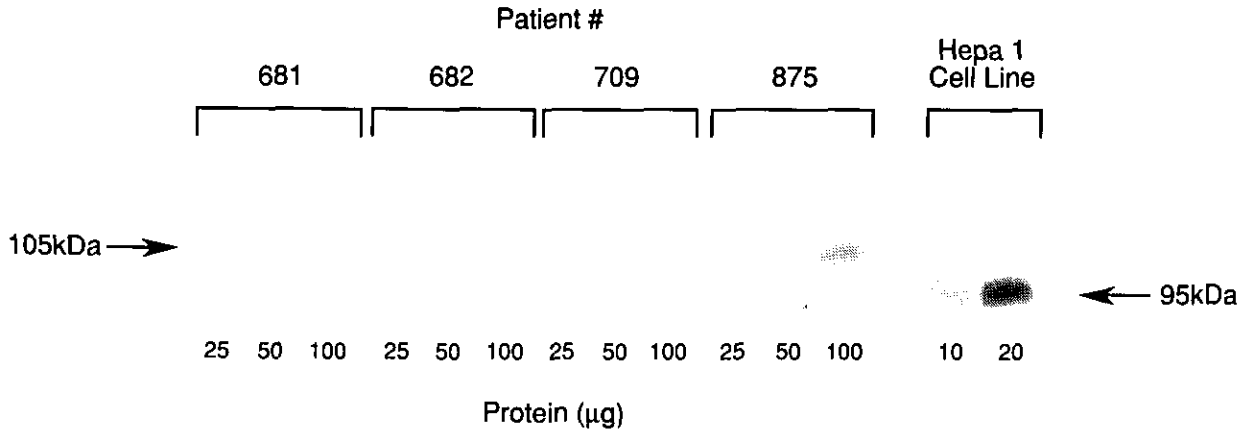

FIGURE 3. Relationship between protein concentration and amount of *Ah* receptor in human lymphocytes. *Ah* receptor determination: cytosols from mitogen-stimulated human lymphocytes were prepared in HEDG buffer containing 1mM phenylmethylsulfonyl fluoride, 2 µg/mL leupeptin, and 20 mM sodium molybolate by centrifugation at 100,000*g*. *Ah* receptor levels were quantified by photoaffinity labeling as previously described by Poland et al. (*45,46*).

tions of the assay. Intra-individual variation in receptor preparations, however, limited our ability to draw conclusions about receptor expression and its contribution to the phenotypic expression of EROD inducibility. We are now investigating factors involved in *Ah* receptor expression in human lymphocytes and whether differences in receptor concentration or affinity contribute to phenotypic inducibility of CYP1A1 activity. Interdividual differences in human CYP1A1 induction by TCDD may reflect both polymorphism in the *CYP1A1* gene as well as differences in the *Ah* receptor itself.

Relevance of Biomarkers to Risk Assessment of TCDD

Considerable controversy surrounds the risk assessment of TCDD and its structural analogs (*11,44*), most of which is related to the selection of risk assessment models. U.S. Federal agencies and countries that use the linear multistage model have established considerably lower acceptable daily intakes (ADIs) than countries that use safety factor/threshold models. For example, the U.S. Environmental Protection Agency's ADI for TCDD is a 6 fg/kg/day, whereas Canada has set an ADI of 10,000 fg/kg/day. These discrepancies, coupled with our growing knowledge of the mechanisms of action responsible for the toxic and biochemical effects of TCDD, have provided impetus for the generation of biologically-based risk assessment models. These models are based on the generally accepted dogma that most, if not all, of the effects of TCDD require interaction with the *Ah* receptor; however, it must be kept in mind that interaction of TCDD with the *Ah* receptor is necessary but not sufficient to produce its effects.

When a simple relationship exists between receptor occupancy and biological response, there is no threshold for response (*14,48*). Such a situation may exist for induction of CYP1A1 and CYP1A2. For example, extrapolation of data on CYP1A1 or CYP1A2 induction in our rat tumor promotion model gives no evidence of a threshold. Therefore, if CYP1A1 is used as a surrogate for TCDD-mediated cancer dose-response relationships, then the ADI (one cancer in 10^6 individuals) would be similar to the current U.S. standard of 6 fg/kg/day (*49*). If a threshold model is employed in which CYP1A1 induction is used as a surrogate for cancer, the following considerations emerge. The current lowest detectable dose for inducing CYP1A1 in rat liver is 100 pg/kg/day (Table 1); we have preliminary data, using a polymerase chain reaction method, that the lowest detectable dose for CYP1A1 induction could be as low as 10 pg/kg/day. If a 100-fold safety factor were employed, the ADI would be 100 fg/kg/day; if a 1000-fold safety factor were used, it would 10 fg/kg/day.

One of the key issues in risk assessment is the relevance of the animal data for estimating human risks. In the case of TCDD, our data provide strong evidence that humans respond similarly to rats for many biochemical effects, including CYP1A1 induction. Although using CYP1A1 induction as a surrogate marker for cancer is simple and attractive, the approach may be flawed. For example, we have shown that dose-response relationships for TCDD-mediated increases in hepatic cell proliferation and preneoplastic lesions are very different from those for CYP1A1 induction. Increases in cell proliferation are detected at much higher doses than those needed to produce enzyme induction (Table 1). Analysis of the rat tumor data suggests that CYP1A1 induction may not be a good predictor of liver tumors. Finally, most TCDD risk assessments have focused on cancer risk, whereas additional toxic responses, such as teratogenesis and immunotoxicity, should also be included in risk assessment models for TCDD.

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